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CS Lab. Mol. Carcinogenesis Clin. Pharmacol. Branch, Natl. Cancer Inst.,
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Activation of a Human Peroxisome Proliferator-Activated Receptor by the Antitumor Agent Phenylacetate and Its Analogs

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Li-Chuan Chen,‡ Talia Sher,*|| Frank J. Gonzalez* and Dvorit Samid‡§¶

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ABSTRACT. The aromatic fatty acid phenylacetate and its analogs induce tumor cytostasis and differentiation in experimental models. Although the underlying mechanisms of action are not clear, effects on lipid metabolism are evident. We have now examined whether these compounds, structurally similar to the peroxisome proliferator clofibrate, affect the human peroxisome proliferator-activated receptor (hPPAR), a homolog of the rodent PPAR α , a transcriptional factor regulating lipid metabolism and cell growth. Gene transfer experiments showed activation of hPPAR, evident by the increased expression of the reporter gene chloramphenicol acetyltransferase linked to PPAR-response element from either the rat acyl-CoA oxidase or rabbit CYP4A6 genes. The relative potency of tested drugs in the co-transfection assay was: 4-iodophenylbutyrate > 4-chlorophenylbutyrate > clofibrate > phenylbutyrate > naphthylacetate > 2,4-D > 4-chlorophenylacetate > phenylacetate >> indoleacetate. Phenylacetylglutamine, in which the carboxylic acid is blocked, was inactive. The ability of the aromatic fatty acids to activate PPAR was confirmed *in vivo*, as CYP4A mRNA levels increased in hepatocytes of treated rats. Further studies using human prostate carcinoma, melanoma, and glioblastoma cell lines showed a tight correlation between drug-induced cytostasis, increased expression of the endogenous hPPAR, and receptor activation documented in the gene-transfer model. These results identify phenylacetate and its analogs as a new class of aromatic fatty acids capable of activating hPPAR, and suggest that this nuclear receptor may mediate tumor cytostasis induced by these drugs. *BIOCHEM PHARMACOL* 52:4:659–667, 1996.

KEY WORDS. aromatic fatty acids; phenylacetate; phenylbutyrate; clofibrate; acyl-CoA oxidase; cytochrome P450IV; cytostasis; nuclear receptors

PA**, a naturally occurring plasma component, is a novel antitumor agent currently in clinical trials. Preclinical data indicate that PA and its related aromatic fatty acid, PB, cause cytostasis and differentiation of various hematopoietic and solid tumors *in vitro* and in animals [1–6]. Phase I studies with PA in the treatment of adults with cancer

showed that drug concentrations effective in experimental models (1–4 mM) can be achieved with no significant toxicity and benefit patients with high-grade gliomas and hormone refractory prostatic carcinoma [7, 8]. Previous experience with individuals suffering from hyperammonemia showed that, like PA, PB is well-tolerated at high concentrations [9, 10].

The promise of PA and PB as anticancer agents has prompted the search for more potent analogs and characterization of their mechanisms of action [11]. Structurally, these compounds share the aromatic nucleus and carboxylic acid features in common with “fibrate” hypolipidemic drugs, such as CF (Fig. 1). A functional link is suggested by the effect of these drugs on lipid metabolism. For example, both PA and CF inhibit cholesterol synthesis [3, 12], cause fatty acid accumulation in cultured tumor cells [2, 3], and induce adipocyte differentiation in embryonic mesenchymal cells [1, 13, 14]. CF belongs to a diverse group of compounds collectively termed PPs, based on their ability to induce the proliferation of peroxisomes in rodent hepatocytes following systemic administration [15]. PPs are

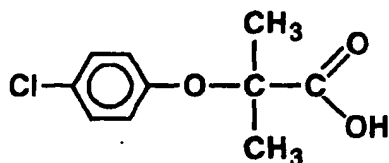
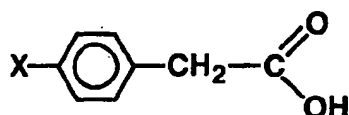
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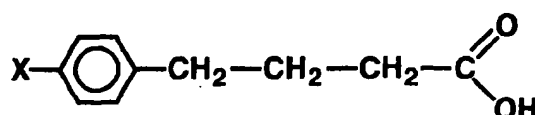
** Abbreviations: ACO, acyl-CoA oxidase; CAT, chloramphenicol acetyltransferase; CF, clofibrate; CPA, 4-chlorophenylacetate; CPB, 4-chlorophenylbutyrate; CYP4A, a member of the P450IV family; cytochrome 2,4-D, 2,4-dichlorophenoxyacetic acid; hPPAR, human PPAR; IAA, indole acetic acid; IPB, 4-iodophenylbutyrate; NA, naphthylacetate; PA, phenylacetate; PAG, phenylacetylglutamine; PB, phenylbutyrate; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; and PPRE, peroxisome proliferator response element.

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(a) Classical peroxisome proliferator: clofibric acid (CF)**(b) Aromatic fatty acids: phenylacetic acid and analogs**

X = H, phenylacetic acid (PA)

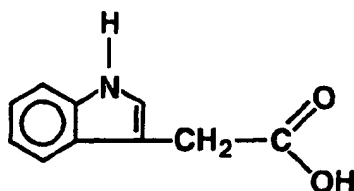
X = Cl, 4-chlorophenylacetic acid (CPA)



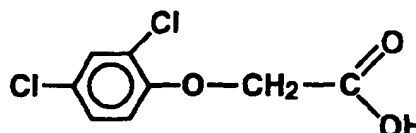
X = H, phenylbutyric acid (PB)

X = Cl, 4-chlorophenylbutyric acid (CPB)

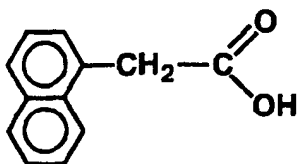
X = I, 4-iodophenylbutyric acid (IPB)



indole acetic acid (IAA)



2,4-dichlorophenoxyacetic acid (2,4-D)



naphthylacetic acid (NA)

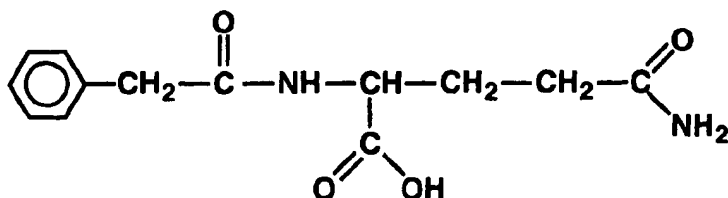
(c) End metabolite of phenylacetate: phenylacetylglutamine (PAG)

FIG. 1. Chemical structures of (a) clofibric acid, (b) tested aromatic fatty acids, and (c) phenylacetylglutamine.

known to activate the nuclear PPARs, which function as ligand-activated transcription factors [16, 17]. PPARs isolated from rodent cells up-regulate the expression of several genes coding for lipid-metabolizing enzymes, including: (a) the peroxisomal β -oxidation enzymes fatty acyl-coA oxi-

dase, enol-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme, and 3-ketoacyl-CoA thiolase [18–22]; (b) enzymes of the peroxisomal cytochrome P450IV family (CYP4A) involved in fatty acid ω -hydroxylation [23]; (c) fatty acid binding proteins [24, 25]; (d)

mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthetase, a key enzyme controlling ketogenesis [26], and medium chain β -acyl-CoA dehydrogenase, a key enzyme in mitochondrial β -oxidation [27]; and (e) malic enzyme, a lipogenic enzyme [28].

hPPAR, a homolog of the mouse PPAR α , was cloned recently and shown to similarly transactivate genes containing PPREs [29]. Sequence homology indicates that hPPAR belongs to the nuclear steroid receptor superfamily, which includes the retinoid, vitamin D, and thyroid hormone receptors, all critical to cell growth and differentiation [16, 30].

In view of the structural and functional similarities between PA and some PPs, as well as the importance of the nuclear receptors to growth control, we tested the hypothesis that PA and its analogs might activate hPPAR, and examined the correlation between receptor activation and tumor cytostasis induced by these aromatic fatty acids.

MATERIALS AND METHODS

Cell Culture and Reagents

Human glioblastoma A172 and prostatic carcinoma PC3 cell lines were obtained from the American Type Culture Collection (Rockville, MD), and the melanoma 1011 Mel line was a gift from Dr. Jeff Weber (NCI, Bethesda, MD). The human tumor lines were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Gibco Laboratories, Grand Island, NY), 100 U/mL penicillin, 100 μ g/mL streptomycin (Sigma Chemical Co., St. Louis, MO), and 2 mM L-glutamine (Biofluids, Rockville, MD). Mouse Hepa 1 cells were cultured in Dulbecco's modified Eagle's medium with the same supplements as mentioned above.

The sodium salts of phenylacetic acid and phenylbutyric acid were provided by the Elan Pharmaceutical Research Corp. (Gainesville, GA). CF [2-(*p*-chlorophenoxy)-2-methylpropionic acid], CPA, NA, IAA, and 2,4-D were purchased from either Sigma or the Aldrich Chemical Co. (Milwaukee, WI). PAG, CPB, and IPB were synthesized as previously described [4, 11]. All chemicals were dissolved in distilled water and neutralized with NaOH as needed.

Plasmids

The hPPAR expression vector, consisting of the *Nru*I-*Bam*HI fragment of the hPPAR, has been described [29]. DNA inserted into pSG5 (Stratagene, La Jolla, CA) includes the rat ACO promoter region (-640 to -472), containing the ACO-PPAR response element [18], 5' to the minimal thymidine kinase promoter driving the CAT gene. The 3xZ-L15CAT reporter plasmid was constructed as previously described [29]. The construct contains three copies of the Z-element of CYP4A6 inserted 5' to the CAT gene promoter (a gift from M. Mitas at the National Institutes of Health, Bethesda, MD). pBluescript II-KS(-) (Stratagene) or pUC19 (Promega, Madison, WI) was used as carrier

DNA in the transfection assays. We used pSV232L Δ 5' (a gift from Dr. S. Subraman, University of California, San Diego, CA), a luciferase expression plasmid [31], as a control for transfection efficiencies and to standardize CAT activities. All plasmids were purified twice on cesium chloride gradient before use for transfection assays.

Transfection and Drug Treatment

Twenty hours before transfection, mouse Hepa 1 cells were transferred into 6-cm dishes at 25–30% confluency in growth medium containing 1.5% charcoal-treated fetal bovine serum (Sigma). Medium was replenished 3 hr before transfection, and the gene transfer was carried out using the calcium phosphate precipitation technique [32]. The plasmid mixture for the co-transfection assays depicted in Fig. 2a contained 1 μ g of ACO-L15CAT, 1 μ g of either pSG5-hPPAR or pSG5 as control, 1.2 μ g of luciferase vector pSV232L Δ 5', and 1.8 μ g of pBluescript II KS(-) as DNA carrier. The final DNA content was 5 μ g per dish in 6 mL medium. The mixture added to each dish for the co-transfection assays depicted in Fig. 2b contained 1 μ g of 3xZ-L15CAT, 1 μ g of pSG5-hPPAR or pSG5 as control, 1.2 μ g of luciferase vector pSV232L Δ 5', and 1.8 μ g of pUC19 as carrier DNA.

Eighteen hours after transfection, the medium was changed, and tested drugs were added at indicated concentrations. After 24 hr of treatment, cells were harvested by scraping and cell pellets were collected. Following five consecutive cycles of freezing and thawing, the supernatants of the cell lysates were assayed for CAT [33] and luciferase [31] activities. After separation by thin-layer chromatography (Baker, Phillipsburg, NJ), the radioactivity of substrate and metabolites was measured with a phosphorimager. CAT activity was normalized to the corresponding luciferase measurements by a Monolight 2010 luminometer (Analytical Luminescent Laboratories, San Diego, CA).

Animal Studies

Female Sprague-Dawley rats (6 months old) were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Rats were given an interperitoneal injection of PA (500 mg/kg), PB (400 mg/kg), CF (500 mg/kg), or IPB (130 mg/kg) 48 hr prior to being killed. Liver samples were removed and total RNA was isolated.

Northern Blot Analysis

Total RNA was extracted from rat liver using a guanidinium thiocyanate-cesium chloride technique [34], and mRNA was obtained from human tumor cells using the FastTrack mRNA isolation kit (Invitrogen, San Diego, CA). The RNA species were separated on 1.2% agarose/formaldehyde denaturing gels and transferred to NaOH capillary membranes (NEN, Boston, MA). Northern blot analysis was performed under high-stringency conditions: RNA was hybridized with specific cDNA probes (see below) at 40° for 18 hr, and the blots were then washed with

0.3× sodium chloride/sodium citrate solution with 0.5% sodium dodecyl sulfate at 60°. Autoradiography was performed, and the radioactive signals corresponding to the hybridized bands were integrated with a phosphorimager. Results obtained with the β -actin probe were used to normalize those obtained with the cDNA probes specific for CYP4A1 [35] or the human PPAR α (a 0.9-kb BsmI-BamHI fragment comprising the 3'-end of the hPPAR coding sequence [29]). Probes were labeled with [32 P]dCTP using a random primed DNA labeling kit (Ready-To-Go, Pharmacia Biotech Inc., Piscataway, NJ).

Cytostasis Assays

The antiproliferative effect of drugs was determined against a panel of tumor cell lines as described previously [2]. Briefly, cells were seeded at a density of 5×10^4 per well in six-well plates (Costar Co., Cambridge, MA), and drugs were added 24 hr later. On day 5 of treatment, cells were detached with trypsin/EDTA and enumerated using a Coulter counter. The IC_{50} values were determined from a concentration-response curve. Cell viability was assessed by trypan blue exclusion. To investigate the relationship between cytostatic activity and potency of PPAR activation, both parameters were expressed in terms of drug concentration. Relative PPAR activation responses were estimated by EC_{2x} , defined as the concentration at which a compound induced ACO expression to a level twice as high as that of control.

RESULTS

Transactivation of Rat ACO and Rabbit CYP4A6 Reporter Plasmids by Human PPAR in Hepa 1 Cells

To determine whether PA and analogs affect PP-inducible genes, pSG5-hPPAR was co-transfected with either the rat ACO or rabbit CYP4A6 reporter plasmids into Hepa 1 cells. The cells were then exposed for 24 hr to various concentrations of tested drugs (see Fig. 1 for chemical structures). CAT activity (normalized for luciferase) indicated that PA, CPA, PB, CPB, IPB, NA, and 2,4-D, like the classical peroxisome proliferator CF, all induced transactivation by hPPAR of rat ACO, albeit to a different degree (Fig. 2a). Tested compounds also affected the rabbit CYP4A6 response element (Fig. 2b). PA, NA, and 2,4-D are plant growth regulators (auxins). While these compounds activated hPPAR, the auxin IAA had no significant effect on the nuclear receptor. No activity was ob-

served with PAG, a metabolite of PA in which the carboxylic acid is blocked through conjugation with glutamine. The relative potency of the tested compounds was: IPB > CPB > CF > PB > NA > 2,4-D > CPA > PA >> IAA and PAG. As previously reported [29], transfection with pSG5 DNA alone had lower basal level of CYP4A6 expression than the pSG5-hPPAR control group (Fig. 2b), which may be due to activation by endogenous ligands.

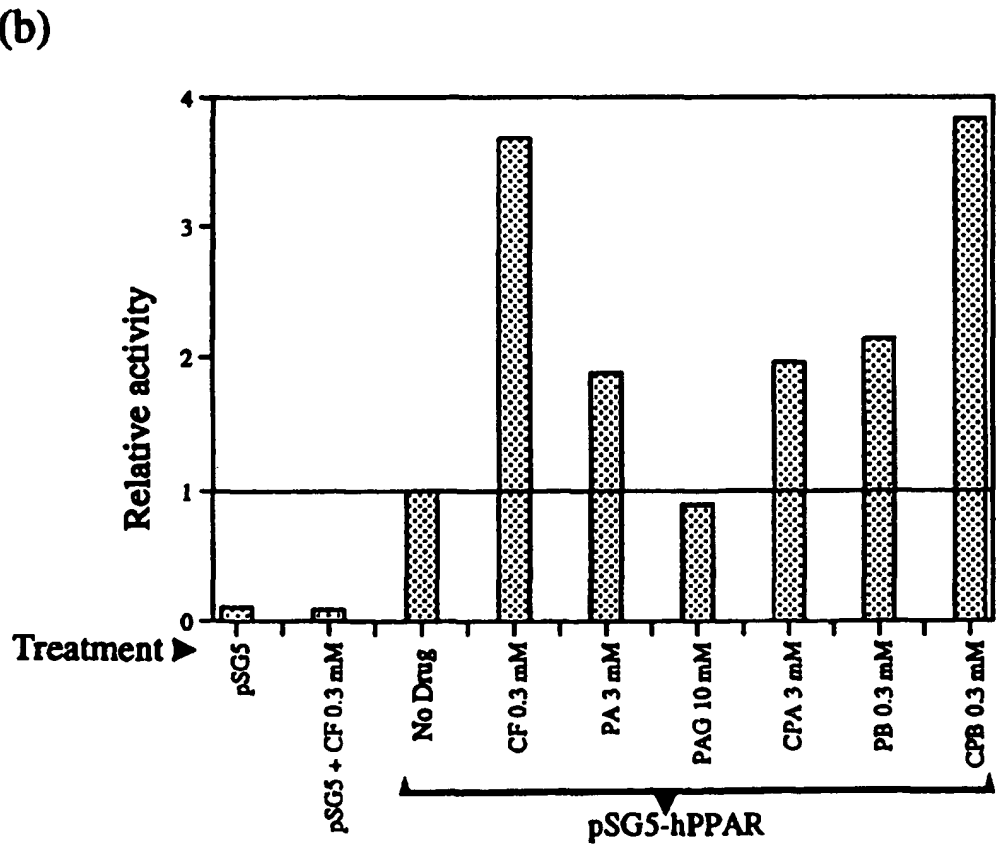
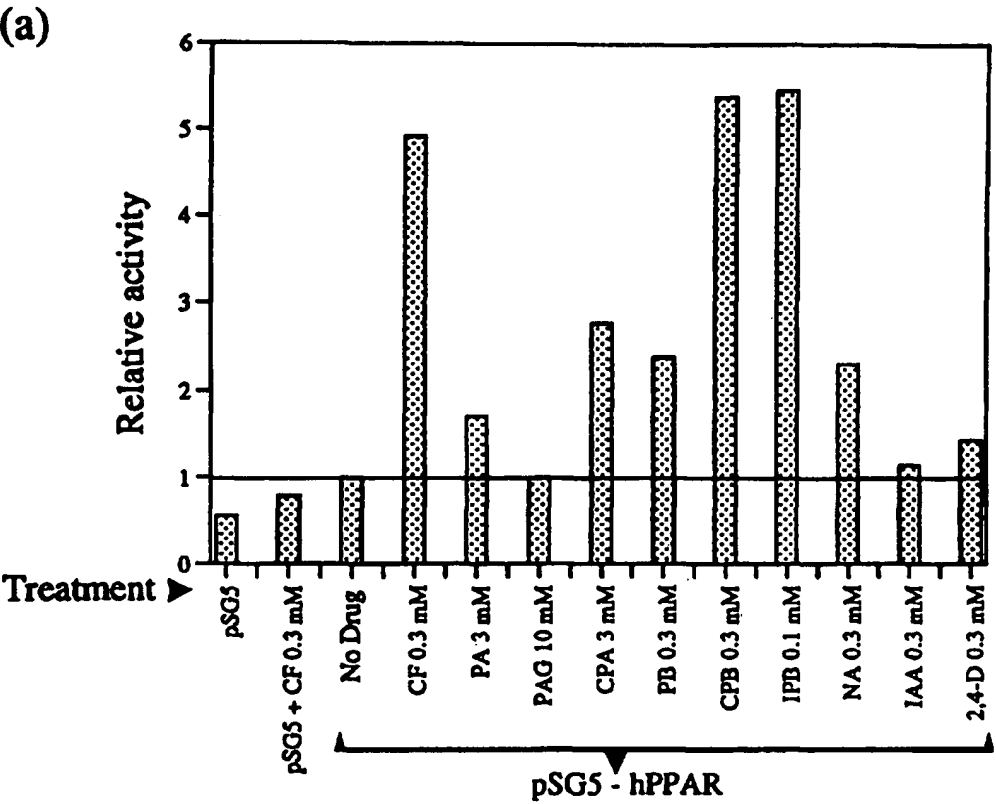
Evidence for PPAR Activation in Rats

To determine whether the aromatic fatty acids activate PPAR *in vivo*, selected compounds including PA, PB, IPB, and CF were administered systemically (i.p.) to rats, and the expression of hepatic CYP4A1 (equivalent of the rabbit CYP4A6) was assessed 48 hr later. Northern blot analysis revealed 1.5 to 2.5-fold higher CYP4A1 mRNA levels in treated animals compared with untreated controls (Fig. 3). In repeated experiments, PA and CF were found to be somewhat more effective than PB and IPB.

Relationship between Drug Effect on hPPAR and Its Antitumor Activity

PA and its analogs are cytostatic agents with selective activity against various human tumor cells [11]. It was of interest, therefore, to examine the relationship between antitumor activity and PPAR activation. First, we determined the cytostatic effect of the aromatic fatty acids on human prostate carcinoma, glioma, and melanoma cells. Marked differences in potency were noted: IPB, the most efficacious activator of PPAR, was found to be the most potent inhibitor of prostatic PC3 cell proliferation, with an IC_{50} of 0.33 mM. PAG, which failed to affect PPAR, was essentially inactive in the cytostasis assay ($IC_{50} > 15$ mM). PA, CPA, NA, PB, CF, and CPB had intermediate IC_{50} values of 4.5, 1.1, 0.92, 0.8, 0.9, and 0.61 mM, respectively. Similar results were obtained for the glioma and melanoma cell lines (data not shown; Ref. 11). Interestingly, there was not only a similar ranking order of efficacy (IPB > CPB > PB > NA = CF > CPA > PA >> PAG), but also a strong correlation between drug-induced cytostasis and the degree of hPPAR activation documented in the co-transfection experiments (Fig. 4). Regression analysis of data summarized in Fig. 4 gave positive values of $r = 0.874$, 0.813, and 0.638 for prostatic, melanoma, and glioblastoma cell lines, respectively. This correlation suggests a potential involvement of PPAR in mediating some of the antitumor effects of PA and related aromatic fatty acids.

FIG. 2. Transactivation of the rat ACO (a) and CYP4A6 promoters (b) by hPPAR in the presence of aromatic fatty acids. (a) Hepa 1 cells were co-transfected with the CAT reporter plasmids containing the rat ACO PPRE (ACO-L15 CAT) together with hPPAR expression plasmid (pSG5-hPPAR). pSG5 served as control. Following transfection, cells were treated for 24 hr with PA, PB, PAG, CF, CPA, CPB, IPB, NA, IAA, and 2,4-D. Results were normalized to the activity of a co-transfected luciferase plasmid (pSV232 Δ 5). The CAT activity obtained on transfection of ACO-L15CAT and pSG5-hPPAR in the absence of drug was taken as 1. The mean values of duplicate determinations from four independent transfections are shown. (b) Hepa 1 cells were co-transfected with the CAT reporter plasmid containing three copies of the CYP4A6 Z-element (3xZ-L15CAT) and either the hPPAR expression plasmid (pSG5-hPPAR) or pSG5 as control. Drug treatment and normalization of the results were identical to that described for Fig. 2a. CAT activity obtained on transfection of 3xZ-L15CAT and pSG5-hPPAR in the absence of drug was taken as 1. The mean values of duplicate determinations from two independent transfections are shown.



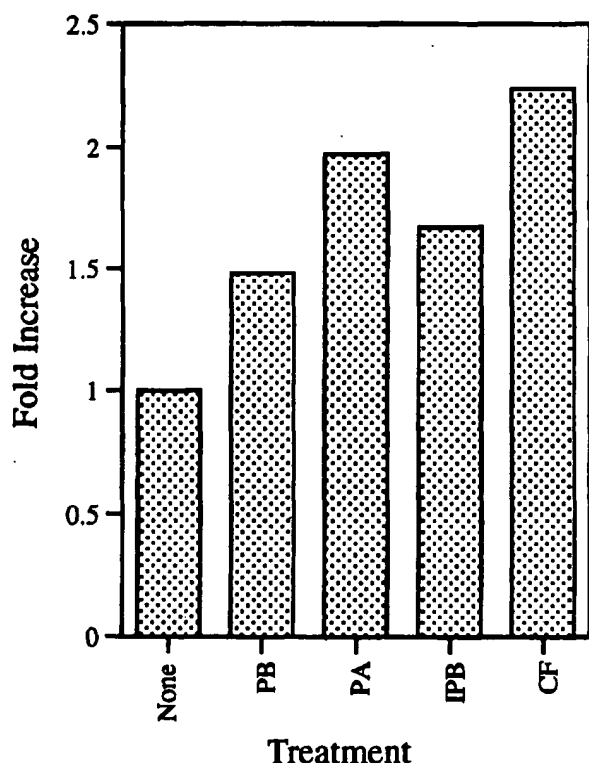


FIG. 3. Induction of CYP4A1 mRNA in liver parenchyma of treated rats. Animals were injected i.p. with 500 mg/kg PA, 400 mg/kg PB, 130 mg/kg IPB or 500 mg/kg CF. The animals were killed 48 hr later, and the livers were removed for RNA analysis as described in Materials and Methods. Quantitative analysis of Northern blots indicates the relative amounts of CYP4A1 mRNA normalized for β -actin.

Increased Expression of the Endogenous hPPAR in Human Tumor Cells Exposed to the Aromatic Fatty Acids

While several genes are known to be transactivated by PPAR α in rodent cells, no such affected genes have been identified in human cells. In the absence of biomarkers, we considered the fact that an activator of PPAR is likely to increase the production of the receptor itself in responsive cells [36]. Northern blot analysis of mRNA isolated from treated human A172 glioma and 1011 melanoma cells showed an increase in hPPAR transcript levels (Fig. 5). Again, the ranking order of drug potency was: CPB > CF > PB > PA (Fig. 5A), i.e. similar to their effect on receptor function (Fig. 2) and tumor proliferation. The increase in hPPAR expression in the human tumor cells was rapid, occurring within 30 min, and stable for at least 5 days of continuous treatment with the aromatic fatty acids (Fig. 5, B and C).

DISCUSSION

The best characterized function of PPARs is the transcriptional regulation of genes involved in the peroxisomal and mitochondrial pathways of lipid metabolism. Various fatty acids (containing more than 6 carbons) and poorly metabolized derivatives thereof are known to regulate their own

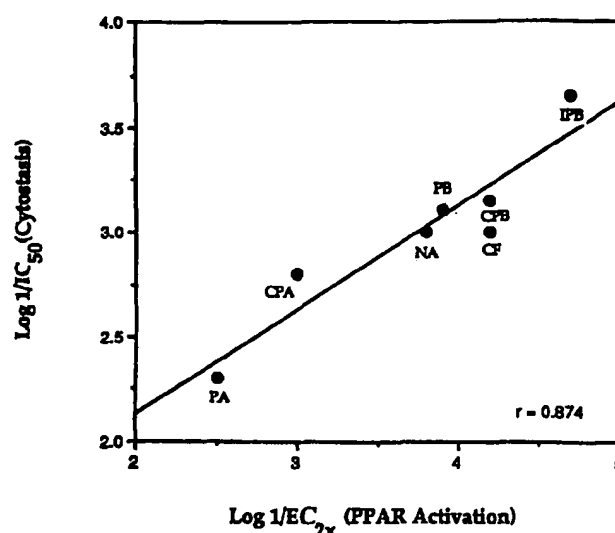


FIG. 4. Correlation between the degree of hPPAR activation in the co-transfection assay and tumor cytostasis induced by PA and its analogs. Relative PPAR activation responses were estimated by EC_{2x}, i.e. drug concentration needed to stimulate ACO expression (in the co-transfection studies) 2-fold over untreated controls. To investigate the relationship between cytostatic activity and potency of PPAR activation, both parameters were expressed in terms of drug concentration. The EC₅₀ values are drug concentrations causing 50% inhibition of prostatic PC3 cell proliferation. The results indicate a linear relationship between hPPAR activation and tumor growth arrest.

metabolism through PPAR activation [37]. We have now identified the aromatic fatty acids PA and its analogs as a new class of antitumor agents capable of activating the human PPAR, a homolog of the rodent PPAR α .

The participation of PPAR in cellular response to PA and analogs was documented *in vitro* and *in vivo*. Gene transfer studies showed transcriptional upregulation of *cis*-elements derived from genes controlling fatty acid β -oxidation (rat ACO) and ω -hydroxylation (rabbit CYP4A6) in cells co-transfected with hPPAR. The relative potency of tested compounds was: IPB > CPB > CF > PB > NA > 2,4-D > CFA > PA >> IAA. PAG, in which the carboxylic acid is blocked, was inactive, indicating that a free carboxylic acid is required for receptor activation. The *in vitro* findings were confirmed in rats, by the increased CYP4A1 mRNA levels in hepatocytes of animals treated systemically with either PA, PB, IPB, or the classical peroxisome proliferator CF. The plasma concentrations of PA in treated rats are in the range of 2–4 mM, which is similar to the pharmacological levels achieved in patients (about 10³-fold above physiological levels) [5, 7, 8]. Such high drug concentrations were required also for PPAR activation *in vitro*. Considering the concentrations needed (millimolar ranges), it is unlikely that PA and analogous aromatic fatty acids act as ligands of the receptor. One possibility is that they displace fatty acids from fatty acid binding proteins, which then activate PPARs [24]. Alternatively, receptor activation may be due to modifications such as phosphorylation [38] or changes in cAMP levels [39].

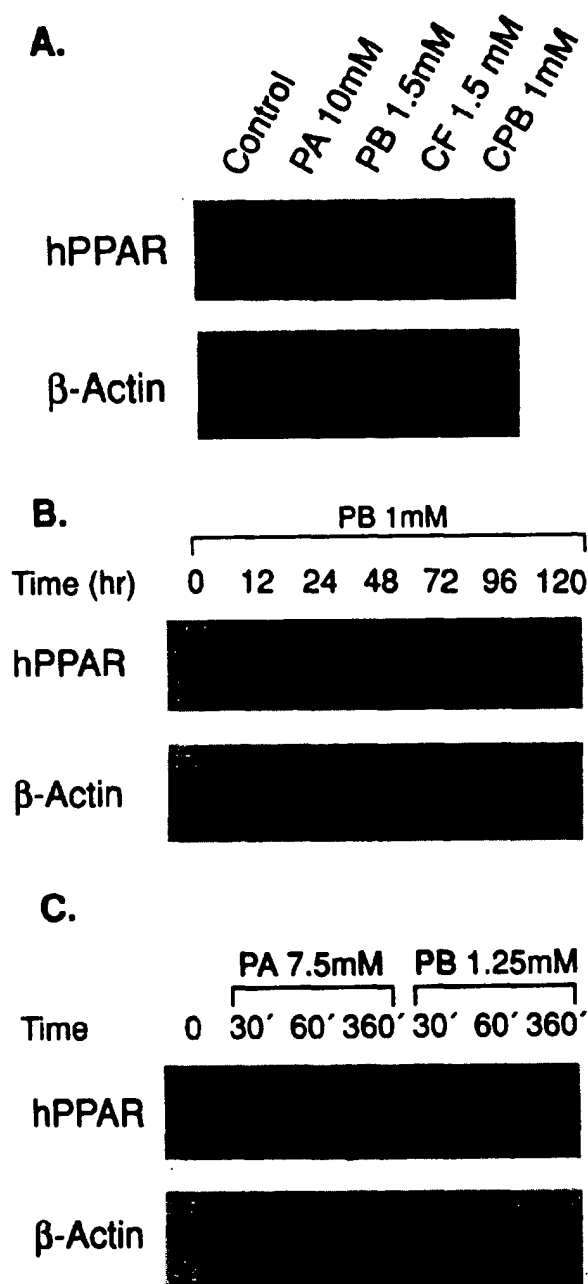


FIG. 5. Increased expression of the endogenous hPPAR in human tumor cells. Shown is a Northern blot analysis of mRNA (5 µg/lane) isolated from human tumor cells. (A) Increased hPPAR expression in A172 glioma cells treated for 3 days with PA and analogs. (B and C) Time-course of hPPAR induction by PB and PA in 1011 melanoma cells. Changes in PPAR levels were evident as early as 30 min after exposure to the aromatic fatty acids, increased further in the first 6 hr, and remained elevated throughout the 5 days of continuous treatment.

PA has antitumor activity in experimental models and humans (see beginning of paper). The finding that PA and its analogs activate PPAR in a gene-transfer model and in rats led us to speculate that the nuclear receptor may mediate some of the antitumor activities of these drugs. In humans, the function of peroxisomes is important for de-

velopment and differentiation, and the number of peroxisomes and the function of peroxisomal enzymes appear to decline during neoplastic transformation [40]. Lowered catalase activity was observed in human breast and colon carcinoma cells compared with the adjacent healthy mucosa, with an inverse relationship between enzyme activity and tumor grade [40]. Since PA, PB, and their derivatives induce phenotypic reversion of malignant human cells of different origins, it was of interest to examine the relationship between antitumor effect and PPAR. Our studies were limited by the fact that, contrary to the well-characterized contribution of PPARs to regulation of gene expression and cell phenotype in rodents, little is known regarding the specific pathways affected in human cells. Unlike in rodents, efforts thus far have failed to show induction of peroxisomes by PPs in cultured human hepatocytes [41–43] and fibroblasts [44]. We and others also could not detect up-regulation in human tumor cells of genes homologous to those induced by PPs in rodent hepatocytes ([45]; our unpublished results). Cross-species comparison may be complicated by a divergence of PPARs throughout evolution, and the ability of members of the steroid nuclear receptor superfamily to form homodimers as well as heterodimers [30]. Nevertheless, several observations have linked PPAR with drug activity against human tumor cells.

Our studies with human glioblastoma, melanoma, and hormone-refractory prostatic adenocarcinoma cells showed a tight correlation between the potency of a compound as an inhibitor of tumor cell proliferation and the degree of hPPAR activation documented in the gene-transfer model. Although it was not possible to demonstrate directly transcriptional transactivation by the endogenous PPAR in the human tumor cells, we considered the fact that agents that activate members of the steroid class of nuclear receptors typically also increase receptor production [36]. Exposure of the human tumor cells to PA and analogs (including the classical peroxisomal proliferator CF) resulted in a rapid induction of hPPAR expression. The levels of hPPAR mRNA increased within 30 min, and persisted for at least 5 days in the presence of continuous treatment. The rapid response of the endogenous hPPAR to PA and PB preceded changes in tumor cell biology (cytostasis and differentiation became detectable after 48 hr), indicating a proximal relationship. In addition to hPPAR, a homolog of mPPAR α , PA and analogs may affect other members of the PPAR gene family. PA is an effective inducer of adipocytosis [1], a differentiation pathway recently linked to activation of PPAR γ [46], and of a fatty acid-activated receptor homologous to the human NUC1 gene [47]. The role of PPARs in drug-induced tumor cytostasis and differentiation is currently under investigation.

As activators of PPARs, PA and its analogs would be expected to affect both fatty acid and cholesterol metabolism. Indeed, profound alterations in lipid metabolism have been documented in cultured cells, in animals, and in humans. For example, cultured tumor cells treated with PA and PB consistently accumulate fat droplets [2], and exhibit

reduced *de novo* cholesterol synthesis and protein prenylation [3, 6, 11]. Similar changes in fatty acids and cholesterol associated with elevated levels of PA were observed in humans and animal models of phenylketonuria [48–50]. A rapid decline in plasma triglyceride levels was also documented in several cancer patients treated with high doses of PA (Thibault A and Samid D, unpublished data).

Considering that PPARs may mediate the antitumor activity of PA and its analogs, other agents capable of activating PPARs should be evaluated as potential anticancer agents as well. The clinical development of some PPs has been hindered by concerns of carcinogenesis based on the experience in rodents, in which induction of peroxisomes is associated with hepatocarcinogenesis. However, the rodent data may have little relevance to human treatment. The long clinical history of human use of such PPs as CF [51], ibuprofen [52], PA and PB [9, 10] proved them safe. Moreover, we have shown recently that PA can benefit some patients with advanced cancers without any significant toxicities [7, 8]. Thus, the present study has implications for the clinical development of some PPs, suggesting that they should not be overlooked simply because of their hepatocarcinogenic effect in rodents.

In conclusion, our findings indicate that PA, PB, and analogous aromatic fatty acids activate the human nuclear receptor analogous to mPPAR α , a transcriptional factor controlling gene expression and cell phenotype. Nuclear receptors have gained much attention recently as targets of selective antineoplastic activity. Activation of retinoic acid receptors (RARs) is thought to be responsible for the impressive clinical activity of all-*trans*-retinoic acid in acute promyelocytic leukemia [53, 54]. Clinical trials with PA have provided preliminary evidence for activity in high-grade glioma, hormone-refractory prostate cancer [7, 8], and B cell malignancies* at well-tolerated doses. We postulate that PPAR activation may contribute to tumor suppression by PA and its analogs, possibly by modulating lipid metabolism and the expression of other critical genes yet to be identified.

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